

DETECTION OF INELASTICALLY-SCATTERED LIGHT WITHIN A FREE STREAM IN AIR IN FLOW CYTOMETRY

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We have recently invented¹ a new technique to collect perpendicular light scatter, both elastic and inelastic, by using the total-internal reflection and optical waveguide properties of a free stream in air (a "flow-stream waveguide"). This produces a 0.88-numerical aperture optical collection and very high optical transmission efficiency to the optical detector. It is easier to align than systems which use microscope objectives and it is less expensive, as well. We extract the light from the aqueous flow stream by placing a conically-polished fiber optic in the stream with the other end of the fiber optic facing an optical detector, with or without wavelength-dispersing optics between the fiber and the detector. We will be reporting our studies of a variety of optical configurations for low-light-level detection, including the use of a small monochromator for spectral analysis. With the small monochromator, we have observed the level of fluorescence of the 3M, Inc. TECS® fiber which is generated by pulses of elastically-scattered light. This, of course, is an undesirable effect. We have also used this system to measure the emission spectrum of fluoresceinated latex beads as part of a collaboration between LLNL and UCSF. For both this collaboration and for internal use for the Genome Project we are examining the limiting features (such as fluorescence of the fiber optics!) with the goal of achieving the detection of weakly fluorescent particles, such as DNA which has undergone digestion by a restriction enzyme.

One unexpected and very pleasant discovery as part of our collaboration with UCSF researchers is a technique to characterize unfixed erythrocytes for A and B surface antigens as well as Rh factor, all performed in a single tube and determined rapidly by flow cytometry.

We have also used the flow-stream-waveguide technique to evaluate a sheath-flow/sample injector which has been fabricated from precision-etched silicon wafers which have been bonded together to create the flow channels. Although it did work the first time we tried it, this microfabricated sheath-flow/sample injector was significantly more difficult to operate than a traditional commercial version.

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¹ Patent application claims allowed by US Patent Office, June 1995.

²Shadi Shakeri, K.S. Venkateswaran, R. Mariella Jr., G.N. Vyas, Human ABO and RH Blood Typing by Simultaneous Three-Color Cytofluorometry, to be presented at the Int. Soc. Blood Transfusion, April 1996, Tokyo